PROLIFERATION AND IMMUNOGLOBULIN SYNTHESIS OF PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH CHRONIC LIVER DISEASE STIMULATED BY STAPHYLOCOCCUS AUREUS COWAN 1 AND INTERLEUKIN 2

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Summary

Proliferation and IgG synthesis of peripheral blood mononuclear cells (PBMC) in response to stimulation with recombinant interleukin 2 (IL-2) and Staphylococcus aureus Cowan 1 (SAC) were evaluated in 32 patients with chronic persistent hepatitis (CPH), chronic active hepatitis (CAH) and liver cirrhosis (LC). Eleven patients had serum HBe antigen, 10 presented with HBe antibody and 11 had non-A, non-B hepatitis. IgG synthesis of PBMC induced with the two stimuli was significantly decreased in patients with CAH and LC when compared with that of controls. However, the generated amounts of IgG were not associated with the HB virus carrier state. B cells and T4+ cells were responsible for the diminished IgG synthesis in patients with CAH and LC when assessed by coculture experiments. On the other hand, proliferative response of PBMC to IL-2 and SAC were similar in controls and patient groups. These findings indicate that IgG production level of PBMC stimulated with IL-2 and SAC can reflect the severity of the underlying disease in chronic hepatitis patients.

Key Words: Chronic liver disease, Ig synthesis, Interleukin 2, Lymphocyte blast transformation, Staphylococcus aureus.

Introduction

The prominence of the mononuclear cells infiltrates in the portal and periportal areas in chronic active hepatitis (CAH) has prompted several studies of cellular immune reactions. Cell-mediated immunity in chronic carriers of hepatitis B virus (HBV) and in patients with CAH has been reported to be abnormal when assessed by in vitro assays such as phytohemagglutinin (PHA)-stimulated lymphocyte blast transformation1,2. The decreased immunoregulation of pokeweed mitogen (PWM)-stimulated immunoglobulin (Ig) synthesis by peripheral blood mononuclear cells (PBMC) in vitro may also be responsible for the continuing liver cell necrosis in CAH3,4.

The T cell growth factor interleukin 2 (IL-2) is a lymphokine produced by T cells stimulated with antigens, mitogens or alloantigens5. Recent studies indicate that IL-2 can play a
role in regulating the proliferation and differentiation of B cells as well as T cells\(^6,7\). Immune interferon (IFN-\(\gamma\)) is also a lymphokine which plays an important role in the immunomodulation\(^8\).

Studies were undertaken to evaluate proliferation and differentiation of PBMC from patients with chronic liver disease, in particular HB surface antigen (HBsAg)-positive patients. Response of PBMC to lymphokines including IL-2 and IFN-\(\gamma\), and stimulus such as Staphylococcus aureus Cowan 1 (SAC) was assessed. SAC has the property of a relatively T-independent B cell activator\(^9,10\). The functions of T and B cells responsible for the altered Ig synthesis in patients with chronic liver disease were also investigated.

**Materials and Methods**

**Patients**

Patients studied consisted of 7 patients with chronic persistent hepatitis (CPH), 13 patients with CAH and 12 patients with liver cirrhosis (LC). The clinical details of the patients are summarized in **Table 1**. Liver diseases were diagnosed on the basis of appropriate clinical, biochemical and histological criteria according to an International Committee\(^11\). HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe were determined with commercially available radioimmunoassay kits (Abbott Laboratories). Thirteen healthy individuals served as controls.

**Lymphocyte preparation**

PBMC were isolated from heparinized venous blood by Ficoll-Hypaque gradient sedimentation. The interphase PBMC were suspended in RPMI 1640 (Grand Island Biological Co., Grand Island, NY) culture medium (RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, glutamine and antibiotics). PBMC were rosetted with neuraminidase-treated sheep erythrocytes (E)\(^12\). The E rosetted and non-rosetted cells were separated by centrifugation on a Ficoll-Hypaque density gradient. Of the rosette forming cells, designated T cells, over 95% formed rosettes with E. Of the interphase cells, designated B cells, less than 3% formed rosettes with E.

Furthermore, to isolate OKT4\(^+\) cell-rich and OKT8\(^+\) cell-rich populations (OKT4\(^+\) defines the inducer/helper subset and OKT8\(^+\) the suppressor/cytotoxic population), OKT4 or OKT8 (Ortho Pharmaceutical corporation) were added to nonfractionated T cell suspensions at a final dilution of 1:150. After 60 min incubation at 37\(^\circ\)C, rabbit sera as a source of complement were added to the cell suspensions at a final dilution of 1:4, and further incubation was performed at 37\(^\circ\)C for 60 min. After washing, the cells were suspended in RPMI 1640 culture medium.

**Additives**

Human IL-2 produced by recombinant DNA technology (rIL-2) with a specific activity of 3.5 \(\times\) 10\(^4\) U/mg protein was supplied from Takeda Pharmaceutical Co. Ltd, Osaka, Japan. It was used at a concentration of 20 ng/ml (0.7 U/ml) for IgG synthesis and 50 ng/ml (1.75

<table>
<thead>
<tr>
<th>Diagnosis (No. studied)</th>
<th>Sex F/M</th>
<th>Age (yr)</th>
<th>SGPT (IU/liter)</th>
<th>(\gamma)-globulin (g/dl)</th>
<th>HBs Ag positive No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic persistent hepatitis (7)</td>
<td>2/5</td>
<td>39±10</td>
<td>56±36</td>
<td>1.0±0.4</td>
<td>5/7</td>
</tr>
<tr>
<td>Chronic active hepatitis (13)</td>
<td>4/9</td>
<td>36±13</td>
<td>36±315</td>
<td>1.4±0.3</td>
<td>9/13</td>
</tr>
<tr>
<td>Liver cirrhosis (12)</td>
<td>2/10</td>
<td>47±11</td>
<td>109±78</td>
<td>1.7±0.5</td>
<td>7/12</td>
</tr>
</tbody>
</table>

All laboratory data were expressed as mean ± SD.